ORIGINAL ARTICLE

Friend leukaemia integration-1 expression in malignant and benign tumours: a multiple tumour tissue microarray analysis using polyclonal antibody

Paulette Mhawech-Fauceglia, Francois R Herrmann, Wiam Bshara, Kunle Odunsi, Luigi Terracciano, Guido Sauter, Richard T Cheney, Jeff Groth, Remedios Penetrante,

J Clin Pathol 2007;**60**:694–700. doi: 10.1136/jcp.2006.039230

See end of article for authors' affiliations

Correspondence to: Dr P Mhawech-Fauceglia, Department of Pathology, Oswell Park Cancer Institute, Elm and Carlton Street, Buffalo, NY 14263, USA; pmhawech1@yahoo.com

Accepted 2 June 2006
Published Online First 17 August 2006

Background: Friend leukaemia integration-1 (FLI-1) antibody is a useful marker for Ewing's sarcoma/primitive neuroectodermal tumour (EWS/PNET) and vascular tumours. However, it is also expressed in subsets of lymphoblastic lymphoma, Merkel cell carcinoma (MCC) and desmoplastic small round cell tumour (DSRCT).

Aim: To determine expression of FLI-1 in various benign and malignant neoplasms, by immunohistochemical analysis on 4323 tumours using multiple tumour microarrays, as well as on whole sections.

Results: FLI-1 was expressed in 46/62 EWS/PNETs, 2/3 olfactory neuroblastomas, 7/102 small cell carcinomas of the lung, 10/34 MCCs, 1/14 rhabdomyosarcoma, 19/132 non-Hodgkin's lymphomas, 2/3 DSRCTs, and in 53/74 benign and malignant vascular tumours. In addition, 27/508 squamous cell carcinomas, 19/837 adenocarcinomas, 10/400 urothelial bladder cancers, 1/40 basal cell carcinomas, 3/29 liposarcomas, 1/40 glioblastoma multiforme and 9/29 medullar carcinomas of the breast expressed FLI-1. The sensitivity and specificity of FLI-1 to distinguish EWS/PNET from all types of malignancies were 74.2% and 96.0%, respectively. Finally, the sensitivity and specificity of FLI-1 to distinguish EWS/PNET from other small round cell tumours (SRCTs) were 74.2% and 91.6%, respectively.

Conclusion: This study was the first to show that FLI-1 can be seen in a variety of solid tumours, some of which had never been explored before. This finding should be kept in mind, especially when using FLI-1 as a marker for finding the primary origin of poorly differentiated metastatic tumour. Finally, despite the expression of FLI-1 in numerous malignancies, it is still considered to be highly sensitive and specific in distinguishing EWS/PNET from other tumour types in general and from other SRCTs in particular.

Ewing sarcoma/primitive neuroectodermal tumour (EWS/PNET) belongs to a group of highly aggressive malignant tumours named small round cell tumours (SRCTs).[1] Almost 80% of EWS/PNET cases present the fusion gene Ewing sarcoma (*EWS*)/*FLI-1* (friend leukaemia integration-1) resulting from the balanced translocation t (11; 22) (q24; q12), which includes the N-terminal transactivation domain of the *EWS* gene and the C-terminus DNA-binding domain of the *FLI-1* gene.[2] FLI-1 antibody is a polyclonal commercially available antibody directed against the C-terminus of FLI-1 protein-binding domain. In normal tissues, FLI-1 was found to be restricted to haematopoietic cells and endothelial cells. FLI-1 was mainly expressed in EWS/PNET with a specificity of over 90%, and later on it was added to CD99 as a useful marker in the histological diagnosis of EWS/PNET.[3 4] However, further studies showed that FLI-1 was frequently seen in various tumour types, including vascular tumours, lymphoblastic lymphoma, Merkel cell carcinoma (MCC) and desmoplastic small round cell tumour (DSRCT).[3–6] Furthermore, few studies on FLI-1 immunoexpression in tumour types other than those mentioned above were found. In those studies, FLI-1 was not expressed in any of the cases analysed.[3–6] However, those studies were hampered by a small number of samples and by a very limited selection of tumour types.[3–6] Hence, the aim of this study is to define the expression of FLI-1 using FLI-1 polyclonal antibody in a large number of benign and malignant tumours. Our results will shed some light on the possibility of FLI-1 expression in a variety of tumours, which could be of major importance when using FLI-1 to evaluate poorly differentiated tumours, especially when they are metastatic and of unknown origin. To accomplish this aim, a multiple tumour microarray technique has been used, which is a high-throughput, efficient and practical technique for evaluating the expression of immunohistochemical markers.[7–9]

MATERIALS AND METHODS

Tissue microarray and immunohistochemistry

A formalin-fixed, paraffin-wax-embedded multiple tumour tissue microarray was used. The microarray was constructed as described previously.[7–10] In addition, whole sections from 26 EWS/PNET, 28 MCC and three DSRCT cases were included in the study. Whole sections from normal tissues were also included. An H&E-stained section was evaluated for the presence of the tumour by light microscopy. Sections of 4 μm thickness were processed for immunohistochemical analysis. Endogenous peroxidase was blocked with 0.3% hydrogen peroxidase for 30 min. Antigen retrieval was carried out in a high pH buffer for 3 min in a steamer/cooker. Subsequently, sections were incubated with FLI-1 polyclonal (C-19) antibody (clone sc-356, 1:50, Santa-Cruz, California, USA) at room temperature for 30 min. A biotin-free horseradish peroxidase enzyme-labelled polymer of the Envision plus detection system was added (Dakocytomation). The diaminobenzidine complex was used as the chromogen detection reagent. In negative

Abbreviations: DSRCT, desmoplastic small round cell tumour; EWS/PNET, Ewing's sarcoma/primitive neuroectodermal tumour; FLI-1, friend leukaemia integration-1; MCC, Merkel cell carcinoma; NHL, non-Hodgkin's lymphoma; ONB, olfactory neuroblastoma; RMS, rhabdomyosarcoma; SRCT, small round cell tumour

Table 1 Friend leukaemia integration-1 immunoexpression in normal human tissues

Tissue	FLI-1 immunoexpression
Endometrium	
Glands	Negative
Stroma	Negative
Myometrium	Negative
Cervix	
Exocervix	Negative
Endocervix	Negative
Ovary	Negative
Fallopian tubes	Negative
Prostate	Negative
Testis	Negative
Bladder	Negative
Kidney	
Tubules	Negative
Glomerulus	Negative
Breast	Negative
Spleen	Positive
LN	Positive
Oesophagus	Negative
Stomach	Negative
Colon	Negative
Gallbladder	Negative
Small intestine	Negative
Pancreas	
Acini	Negative
Islets	Negative
Liver	Negative
Lung	
Pneumocytes	Negative
Bronchus	Negative
Heart	Negative
Endothelium	Positive
Skin	Negative
Adipocytes	Negative
Smooth muscle	Negative
Salivary glands	Negative
Thyroid	Negative
Parotid	Negative
Parathyroid	Negative
Ganglia cells in the GI tract	Negative
Nerve	Negative
Brain	Negative

FLI-1, Friend leukaemia integration-1; GI, gastrointestinal; LN, lymph node

controls, a normal rabbit serum was used instead of the primary antibody. Nuclear staining was required in order to consider the FLI-1 staining positive. Evaluation of the immunohistochemistry slides was performed semiquantitatively by two pathologists (PM-F and WB), who were not aware of the original histological diagnosis, using a double-head microscope. The scores were reviewed, and, whenever a discrepancy was noted between the first and second readings, a third pathologist (RP) was asked to review the cases. The three pathologists reached an agreement on the final scoring. For scoring, intensity and percentage of positive cells were taken into consideration. The intensity was classified into three categories: weak, moderate and strong. A cut-off of $\geqslant$5% positive tumour cells was used to define positive results, as described previously.[4] However, all cases had >50% positive tumour cells.

RESULTS

Table 1 summarises FLI-1 immunoexpression in normal human tissues. In normal tissues, FLI-1 expression was restricted to haematopoietic cells and endothelial cells. It was negative in all other normal tissues.

Table 2 gives the summary of FLI-1 expression in 4323 benign and malignant tumour types.

FLI-1 expression in the SRCT group was as follows: 46/62 (46.8%) EWS/PNET, 10/34 (29.4%) MCC, 7/102 (6.9%) small cell carcinoma of the lung, 19/132 (14.4%) non-Hodgkin's lymphoma (NHL), 1/14 (7.1%) rhabdomyosarcoma (RMS), 2/3 (66.7%) DRSCT and 2/3 (66.7%) olfactory neuroblastoma (ONB) cases. FLI-1 was expressed in 53/74 (71.6%) of all vascular neoplasms, including 3/4 (75.0%) angiosarcoma, 19/29 (65.5%) Kaposi's sarcoma, 7/12 (58.3%) haemangiopericytoma and 24/29 (82.7%) capillary haemangioma. In addition, there were 27/508 (5.3%) squamous cell carcinomas, 19/837 (2.3%) adenocarcinomas, 10/400 (2.5%) urothelial carcinomas of the bladder, 3/29 (10.3%) liposarcomas, 1/40 (2.5%) glioblastoma multiforme and 1/40 (2.5%) basal cell carcinoma of the skin (fig 1A–D). Furthermore, 9/29 (31.0%) medullary carcinomas of the breast showed immunoexpression of FLI-1. In more than half of the negative MCC cases there was a cytoplasmic background staining, although, once again, those cases were interpreted as negative, owing to the absence of nuclear staining for FLI-1 (fig 2).

Table 3 illustrates the sensitivity, specificity and predictive value of FLI-1 in distinguishing EWS/PNET from all other malignancies, as well as from tumours belonging to the SRCTs and others that might enter in the differential diagnosis with EWS/PNET.

We found FLI-1 to be highly sensitive in distinguishing EWS/PNET from all types of malignancies in general, and from germ cell tumours, as well as from other tumours belonging to the group of SRCTs in particular, with a value of 74.2% for each. On the other hand, FLI-1 was highly specific in distinguishing EWS/PNET from all types of malignancies in general, and from germ cell tumours, as well as from other SRCTs in particular, with values of 96.0%, 100% and 66.7%, respectively. FLI-1 had a low positive predictive value (25.6%) in distinguishing EWS/PNET from all other solid tumours, but had a very high negative predictive value of 99.5%. On the other hand, FLI-1 had a high positive predictive value (100%), as well as negative predictive value (90.2%), in distinguishing EWS/PNET from germ cell tumours. Finally, the positive and negative predictive values of FLI-1 to distinguish EWS/PNET from other SRCTs were 66.7% and 94.0%, respectively.

DISCUSSION

EWS/PNET is a small, blue, round cell tumour with a very characteristic t(11,22) chromosomal rearrangement, which results in fusion of the EWS and FLI-1 genes. The resultant fusion protein promotes oncogenesis, which is necessary for continued growth of tumour cell lines. Studies show that loss of the COOH-terminal domain can attenuate the ability of EWS/FLI-1 to promote anchorage-independent growth. Furthermore, cells with EWS/FLI-1 COOH deletion mutants show a progressive decrease in tumour oncogenesis and loss of round cell morphology on histological examination. The above data led to the suggestion that the C-terminus of FLI-1 seems to have a crucial functional role in EWS/FLI-1 oncogenicity.[3] Since the commercial availability of FLI-1, few comprehensive studies on its expression in benign and malignant tissues have been published. In 1999, Nilsson *et al*[11] were the first to demonstrate FLI-1 expression using sc-356 polyclonal antibody in EWS/PNET cell lines and in all five formalin-fixed paraffin-wax-embedded EWS/PNET cases. Soon after, Folpe *et al*[4] explored its expression in a series of SRCTs. They showed that FLI-1 was expressed not only in 71% EWS/PNET but also in various SRCTs, including 7/8 (88%) lymphoblastic lymphomas, 1/1 DSRCT, 0/1 poorly differentiated synovial sarcoma, 0/32 RMS, 0/3 neuroblastoma, 0/3 Wilms' tumour, 0/8 ONB and 0/1 mesenchymal chondroblastoma, indicating that FLI-1 can be expressed in a variety of SRCTs but its expression is still highly

Table 2 Summary of Friend leukaemia integration-1 immunoexpression in benign and malignant tumours

Organs/tumour type	Negative	Weak +	Moderate +	Strong +	Total
Squamous cell carcinomas					
Total squamous cell carcinoma	481	13	10	4	508
Head and neck	206	6	6	2	220
Skin	143	4	2	1	150
Skin—undefined source	51	2	2	0	55
Anus	5	0	0	0	5
Vulva	42	1	0	0	43
Penis	45	1	0	1	47
Gynaecological	52	1	0	0	53
Cervix	47	1	0	0	48
Vagina	5	0	0	0	5
Oesophagus	36	0	0	0	36
Lung	44	2	2	1	49
Adenocarcinomas					
Total adenocarcinomas	818	5	1	13	837
GI tract	126	2	1	3	132
Upper GI	73	0	1	1	75
Oesophagus	8	0	0	0	8
Stomach	65	0	1	1	67
Intestinal type	43	0	0	1	44
Diffuse type	22	0	1	0	23
Lower GI	53	2	0	2	57
Small intestine	11	0	0	0	11
Colon	42	2	0	2	46
Gallbladder	29	0	0	1	30
Pancreas	50	0	0	0	50
Lung	49	1	0	0	50
Breast	184	0	0	0	184
Lobular	62	0	0	0	62
Ductal	69	0	0	0	69
Mucinous	26	0	0	0	26
Tubular	27	0	0	0	27
Female genital tract	225	2	0	9	236
Endometrium	93	0	0	2	95
Endometrioid	72	0	0	0	72
Serous	21	0	0	2	23
Ovary	130	2	0	7	139
Endometrioid	45	1	0	4	50
Serous	65	1	0	3	69
Mucious	20	0	0	0	20
Uterine cervix	2	0	0	0	2
Genitourinary tract	148	0	0	0	148
Prostate	143	0	0	0	143
Urinary bladder	5	0	0	0	5
Salivary gland adenocarcinoma	7	0	0	0	7
Germ cell tumours					
Total germ cell tumours	148	0	0	0	148
Testis	145	0	0	0	145
Seminoma	67	0	0	0	67
Non-seminoma	78	0	0	0	78
Ovarian germ cell	3	0	0	0	3
Neuroendocrine tumours					
Total neuroendocrine tumours	225	7	6	4	242
Small cell carcinoma of the lung	95	2	3	2	102
Pheochromocytoma	35	0	0	0	35
Paraganglioma	10	0	0	0	10
Carcinoid	52	0	0	0	52
Merkel cell carcinoma of skin	24	5	3	2	34
Small cell carcinoma of urinary bladder	9	0	0	0	9
Malignant soft tissue tumours					
Total malignant soft tissue tumours	148	17	7	5	177
Leiomyosarcoma	40	0	0	0	40
Kaposi's sarcoma	10	13	4	2	29
Malignant fibrohistiocytoma	28	1	0	0	29
Liposarcoma	26	1	0	2	29
Angiosarcoma	1	2	0	1	4
Synovial sarcoma	3	0	0	0	3
Fibrosarcoma	9	0	0	0	9
Rhabdomyosarcoma	13	0	1	0	14
Malignant Schwannoma	10	0	0	0	10
DFSP	4	0	0	0	4
Endometrial stromal tumour	3	0	0	0	3
Desmoplastic small round cell tumour	1	0	2	0	3
PNET	16	17	4	25	62
Brain tumours					
Total brain tumours	131	1	2	0	134
Glioblastoma multiforme	38	1	1	0	40
Astrocytoma	49	0	0	0	49

Table 2 Continued

Organs/tumour type	Negative	Weak +	Moderate +	Strong +	Total
Oligodendroglioma	30	0	0	0	30
Ependymoma	12	0	0	0	12
Olfactory neuroblastoma	2	1	1	0	3
Other tumour types					
Thyroid	89	0	0	0	89
Follicular	38	0	0	0	38
Papillary	51	0	0	0	51
Kidney	157	0	0	0	157
RCC	74	0	0	0	74
Papillary	62	0	0	0	62
Chromophobe	21	0	0	0	21
Salivary gland	59	0	0	0	59
Mucoepidermoid carcinoma	6	0	0	0	6
Adenoid cystic carcinoma	53	0	0	0	53
Urothelial carcinoma of the bladder	390	6	3	1	400
Hepatocellular carcinoma	37	0	0	0	37
DCIS of breast	8	0	0	0	8
Basal cell carcinoma of skin	39	1	0	0	40
Lymphoepithelial carcinoma of pharynx	5	0	0	0	5
Medullary carcinoma of breast	20	1	4	4	29
Undifferentiated carcinoma of oesophagus	1	0	0	0	1
Undifferentiated carcinoma of salivary gland	5	0	0	0	5
Anaplastic carcinoma of thyroid	7	0	0	0	7
Malignant mesothelioma	27	0	0	0	27
Uterus MMMT	4	0	0	0	4
Melanoma of skin	52	0	0	0	52
Melanoma of uvea	48	0	0	0	48
Medulloblastoma	5	0	0	0	5
Retinoblastoma	41	0	0	0	41
Neuroblastoma	31	0	0	0	31
Wilms' tumour	47	0	0	0	47
Haematopoietic neoplasms					
Hodgkin's lymphoma	55	0	0	0	55
Hodgkin—nodular sclerosis	36	0	0	0	36
Hodgkin – mixed	19	0	0	0	19
Non-Hodgkin's lymphoma (NHL)	112	8	2	7	129
MALT lymphoma	51	0	0	0	51
Follicular lymphoma	3	0	0	2	5
CLL	3	0	0	0	3
Burkitt's lymphoma	0	0	0	2	2
Mantel cell lymphoma	1	0	0	0	1
Diffuse large B cell lymphoma	24	4	1	3	32
NHL—unspecified	30	4	1	0	35
TCL	1	0	0	2	3
CML	5	0	0	0	5
Premalignant entities					
Colon dysplasia	132	0	0	0	132
Mild	45	0	0	0	45
Moderate	48	0	0	0	48
Severe	39	0	0	0	39
Cervical CIN III	25	0	0	0	25
Benign soft tissue tumours					
Total benign soft tissue tumours	305	25	6	2	338
GIST	79	0	0	0	79
Benign fibrohistiocytoma	30	0	0	0	30
Glomus tumour	10	0	0	0	10
Granular cell tumour	8	0	0	0	8
Tendon sheet, giant cell tumour	33	2	0	0	35
Haemangiopericytoma	5	6	1	0	12
Capillary haemangioma	5	17	5	2	29
Neurofibroma	43	0	0	0	43
Leiomyoma	61	0	0	0	61
Lipoma	31	0	0	0	31
Benign entities					
Thyroid adenoma	46	0	0	0	46
Parathyroid adenoma	40	0	0	0	40
Adrenal gland adenoma	15	0	0	0	15
Salivary gland pleomorphic adenoma	46	0	0	0	46
Warthin's tumour	28	0	0	0	28
Kindey—oncocytoma	23	0	0	0	23
Thymus—thymoma	24	0	0	0	24
Ovary—Brenner tumour	8	0	0	0	8
Optic glioma	1	0	0	0	1
Craniopharyngeoma	3	3	0	0	6
Ganglioneuroma	7	0	0	0	7
Meningioma	49	0	0	0	49
Schwannoma	46	0	0	0	46
Breast phylloides tumour	13	0	0	0	13

Table 2 Continued

Organs/tumour type	Negative	Weak +	Moderate +	Strong +	Total
Mesothelial adenomatoid tumour	9	0	0	0	9
Skin, benign appendages tumour	29	0	0	0	29
Skin, benign nevus	46	0	0	0	46

CIN, cervical intraepithelial neoplasia; CLL, chronic lymphocytic leukaemia; CML, chronic myelogenous leukaemia; DCIS, ductal carcinoma in situ; DFSP, dermatofibrosarcoma protuberans; GI, gastrointestinal; GIST, gastrointestinal stromal tumour; MALT, mucosa-associated lymphoid tissue; MMMT, malignant mixed mullerian tumour; NHL, non-Hodgkin's lymphoma; PNET, primitive neuroectodermal tumour; RCC, renal cell carcinoma; TCL, T-cell lymphoma.

specific (90%) for EWS/PNET. Since then, FLI-1 has been used as a marker for EWS/PNET, and its use has been repeatedly described in numerous case reports.[12–15] Another work, by Llombart-Bosch *et al*,[3] found FLI-1 expression in 16/19 (84%) EWS/PNETs, 4/5 (80%) NHLs (large cell type, unspecified lineage), 2/9 (22.2%) neuroblastomas and in 3/6 (50%) undifferentiated synovial sarcomas. In our series of SRCTs, FLI-1 was found in 46/62 (74.2%) EWS/PNETs, 2/3 (66.7%) ONBs, 2/3 (66.6%) DSRCTs, 1/14 (7.1%) RMS and 7/102 (6.9%) small cell carcinomas of the lung. In addition, and similar to the results by Folpe *et al*, we found FLI-1 to be almost always negative in Wilms' tumours (n = 47) and neuroblastomas (n = 31), findings that are both important and very useful in the differential diagnosis of SRCTs in children. Finally, FLI-1 was negative in our series of 148 cases of germ cell tumours, which might enter into the differential diagnosis with EWS/PNET. From these data, we concluded that FLI-1 is a highly sensitive and specific marker in distinguishing EWS/PNET from other SRCTs, as well as from germ cell tumours. Further, we showed that FLI-1 has high positive predictive and negative predictive values in distinguishing EWS/PNET from other SRCTs, as well as from germ cell tumours. Also, 19/132 (13.1%) NHLs expressed FLI-1, and it was seen in different NHL subtypes, including follicular, Burkitt's, diffuse large B-cell and peripheral T-cell lymphoma. Thus, FLI-1 is seen not only in lymphoblastic lymphoma but also in other lymphoma subtypes. FLI-1 is expressed in normal endothelial cells, and the *FLI-1* gene has been reported to play an important role in the embryological development of blood vessels.[16] The purpose of

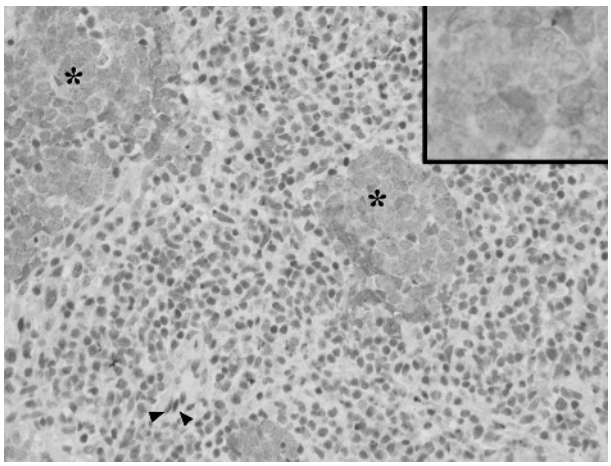

Figure 2 A case of Merkel cell carcinoma negative for Friend leukaemia integration-1 (FLI-1) (tumour cells are indicated by asterisks). The lymphocytic infiltrates and endothelial cells of blood vessels (arrowheads) were positive for FLI-1 and served as internal control.

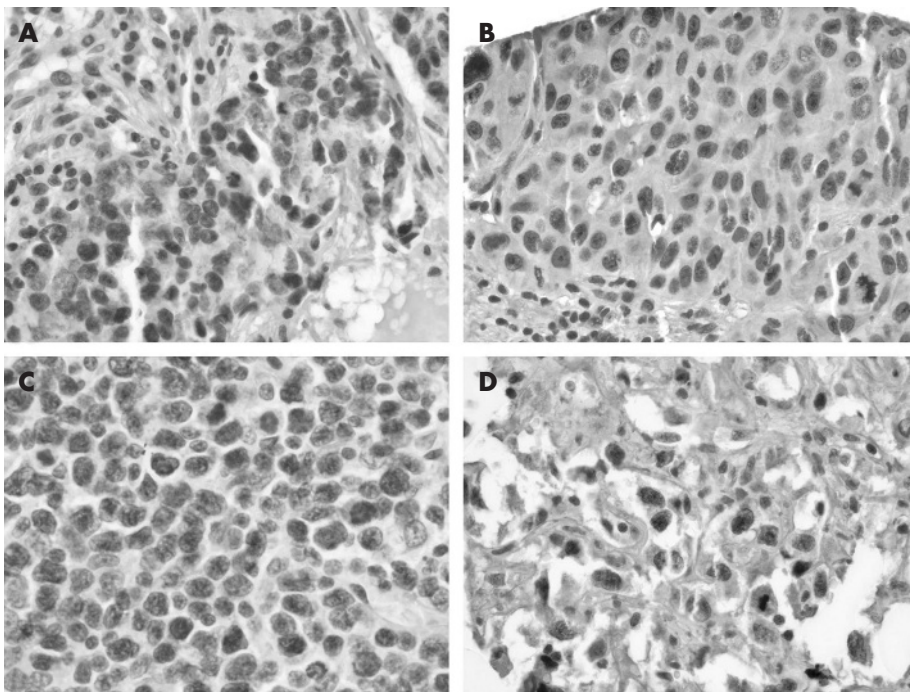

Figure 1 Friend leukaemia integration-1 expression in various tumour types. (A) Squamous cell carcinoma. (B) Medullary carcinoma of the breast. (C) Diffuse large B cell lymphoma. (D) Angiosarcoma.

Table 3 Sensitivity, specificity and predictive value of Friend leukaemia integration-1 in distinguishing Ewing's sarcoma/primitive neuroectodermal tumour (EWS/PNET) from all other malignancies and from tumours belonging to the small round cell tumours and others that might enter in the differential diagnosis with EWS/PNET

	Test					Predictive value of	
Tumour type	Positive	Negative	Total	Sensitivity (95% CI)	Specificity (95% CI)	Positive test (95% CI)	Negative test (95% CI)
EWS/PNET	46	16	62				
All types of malignancies	134	3196	3330	0.742 (0.615 to 0.845)	0.960 (0.953 to 0.966)	0.256 (0.194 to 0.326)	0.995 (0.992 to 0.997)
Germ cell tumours	0	148	148	0.742 (0.615 to 0.845)	1.000	1.000	0.902 (0.846 to 0.943)
Other SRCTs*	23	254	277	0.742 (0.615 to 0.845)	0.916 (0.854 to 0.936)	0.667 (0.543 to 0.776)	0.940 (0.886 to 0.958)

SRCTs, small round cell tumours.

using FLI-1 in the diagnosis of vascular tumours was explored, and FLI-1 seemed to be expressed in 94% of cases.[5 17] In the present study, we found FLI-1 to be expressed in 71.6% of all vascular neoplasms.

There are two studies in the literature discussing FLI-1 expression in tumours other than SRCTs and vascular neoplasms. In the first report, all 60 cases of non-vascular tumours were negative for FLI-1 expression. These cases were 0/16 sarcoma, 0/7 melanoma and 0/45 overall carcinoma, including 3 squamous cell carcinomas (SCC), 12 breast ductal carcinomas, 21 adenocarcinomas (pancreatic (n = 4), pulmonary (n = 5), ovarian papillary serous (n = 5), uterine endometrioid (n = 6) and colonic (n = 1)), 3 renal cell carcinomas, 1 hepatocellular carcinoma, 3 salivary mucoepidermoids and 2 pulmonary carcinoids. In the second report, however, FLI-1 monoclonal antibody (GI-46-222, BD Pharmingen) was used, and the results were as follows: all EWS/PNET (n = 15) and vascular tumour (n = 45) cases, 2/5 MCCs and 1/10 malignant melanoma (MM) were strongly positive for FLI-1, weak expression was seen in 3/5 MCCs, 3/10 synovial sarcomas, 5/10 malignant melanomas, 6/10 lung adenocarcinomas, 1/10 breast carcinoma, and all DSRCTs (n = 5), RMS (n = 10), high-grade pleomorphic sarcomas (n = 10) and colon carcinomas (n = 10) were negative for FLI-1.[18] On exploring FLI-1 expression in a large number of tumours, we found it to be present in a small percentage of a variety of solid tumours, such as 27/508 (6.9%) SCCs, 19/837 (2.3%) adenocarcinomas, 10/400 (2.5%) urothelial bladder carcinomas, 9/29 (31.0%) medullary carcinomas of the breast, 1/40 (2.5%) glioblastoma multiforme and 1/40 (2.5%) basal cell carcinomas of the skin. Despite its expression in a variety of malignant tumours, FLI-1 is still a highly sensitive and specific marker to distinguish EWS/PNET from all types of malignancies. Furthermore, and in this context, it did not have a high positive predictive value but still showed a high negative predictive value. Thus, our study is the first to evaluate a large series of types of tumours using FLI-1 polyclonal antibody, and its expression by the tumours described above should be kept in mind when using FLI-1 as a marker to find the primary origin of a metastatic poorly differentiated tumour.

Finally, although Llombart *et al* found FLI-1 expression in 18/20 (90%) MCCs, we found it in 10/34 (29.4%) MCCs. The difference between the two studies could not be due to difference in techniques; it might be because FLI-1 was carried out on whole sections, and because the samples were interpreted by three pathologists blinded to the tumour types being evaluated. After nuclear expression of FLI-1 was scored by the pathologists, the samples were re-evaluated in light of the discrepancy between Llombart's study and ours. In our results, the majority of MCC cases showed negative nuclear staining for FLI-1 (fig 2).

We found FLI-1 polyclonal antibody in a variety of tumours including EWS/PNET, ONB, small cell carcinoma of the lung, NHL (B and T cell), RMS, DSRCT, vascular tumours and MCB, and subsets of cases of MCC, SCC, adenocarcinomas, urothelial carcinomas and basal cell carcinoma. In conclusion, and despite the expression of FLI-1 in a variety of malignancies, it is still considered to be highly specific and sensitive marker in distinguishing EWS/PNET from other tumours. However, we should be aware of its expression in the above tumour types whenever this marker is used.

Take-home messages

- FLI-1 can be expressed by a wide variety of tumours, where some have not been explored before.
- FLI-1 is still highly sensitive and specific marker to distinguish Ewing's sarcoma/primitive neuroectodermal tumour (EWS/PNET) from other types of malignancies, but with a low positive predictive value (PPV) and good negative predictive value.
- FLI-1 is highly sensitive and specific to differentiate EWS/PNET from germ cell tumours, as well as from other SRCTs with good PPV and NPV.

ACKNOWLEDGEMENTS

We thank Charles LeVea, MD for his critical review of the manuscript. We also thank Mrs Joan Natiella for her histopathological skills, Mr Tim Dolan for his help in searching the archives and Mr Doug Nixon for his illustration expertise.

Authors' affiliations

Paulette Mhawech-Fauceglia, Wiam Bshara, Kunle Odunsi, Richard T Cheney, Jeff Groth, Remedios Penetrante, Department of Pathology and Laboratory Medicine, Roswell Park Cancer Institute, Buffalo, New York, USA
Francois R Herrmann, Department of Rehabilitation and Geriatrics at Geneva University Hospitals, Geneva, Switzerland
Luigi Terracciano, Institute of Pathology, Basel University Hospital, Basel, Switzerland
Guido Sauter, Department of Pathology, University Medical Center Hamburg, Eppendorf, Hamburg, Germany

Competing interests: None declared.

REFERENCES

1 **Devoe K**, Weidner N. Immunohistochemistry of small round-cell tumors. *Semin Diagn Pathol* 2000;**17**:216–24.
2 **Arvand A**, Denny CT. Biology of EWS/ETS fusions in Ewing's family tumors. *Oncogene* 2001;**20**:5747–54.
3 **Llombart-Bosch A**, Navarro S. Immunohistochemical detection of EWS and FLI-1 proteins in Ewing sarcoma and primitive neuroectodermal tumors: comparative analysis with CD99 (MIC-2) expression. *Appl Immunohistochem Mol Morphol* 2001;**9**:255–60.
4 **Folpe AL**, Hill CE, Parham DM, *et al*. Immunohistochemical detection of FLI-1 protein expression. A study of 132 round cell tumors with emphasis on CD99-

positive mimics of Ewing's sarcoma/primitive neuroctodermal tumor. *Am J Surg Pathol* 2000;**24**:1657–62.
5 **Folpe AL**, Chand EM, Goldblum JR, *et al.* Expression of FLI-1, a nuclear transcription factor, distinguishes vascular neoplasms from potential mimics. *Am J Surg Pathol* 2001;**25**:1061–6.
6 **Llombart B**, Monteagudo C, Lopez-Guerrero JA, *et al.* Clinicopathological and immunohistochemical analysis of 20 cases of merkel cell carcinoma in search of prognostic markers. *Histopathology* 2005;**46**:622–34.
7 **Lugli A**, Forster Y, Hass P, *et al.* Calretinin expression in human normal and neoplastic tissues: a tissue microarray analysis on 5233 tissue samples. *Hum Pathol* 2003;**34**:994–1000.
8 **Went P TH**, Lugli A, Meier S, *et al.* Frequent Epcam protein expression in human carcinomas. *Hum Pathol* 2004;**35**:122–8.
9 **Lugli A**, Tornilo L, Mirlacher M, *et al.* Hepatocyte paraffin 1 expression in human normal and neoplastic tissues. Tissue microarray analysis on 3940 tissue samples. *Am J Clin Pathol* 2004;**122**:721–7.
10 **Kononen J**, Bubendorf L, Kallioniemi A, *et al.* Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 1998;**4**:844–7.
11 **Nilsson G**, Wang M, Wejde J, *et al.* Detection of EWS/FLI-1 by immunostaining. An adjunctive tool in diagnosis of Ewing's sarcoma and primitive neuroectodermal tumour on cytological samples and paraffin-embedded archival material. *Sarcoma* 1999;**3**:25–32.
12 **Jimenez RE**, Folpe AL, Lapham RL, *et al.* Primary Ewing's sarcoma/primitive neuroectodermal tumor of the kidney. *Am J Surg Pathol* 2002;**26**:320–7.
13 **Folpe AL**, Goldblum JR, Rubin BP, *et al.* Morphologic and immunophenotypic diversity in Ewing family tumors. A study of 66 genetically confirmed cases. *Am J Surg Pathol* 2005;**29**:1025–33.
14 **Gardner LJ**, Ayala AG, Monteforte HL, *et al.* Ewing sarcoma/peripheral primitive neuroectodermal tumor. *Appl Immunohistochem Mol Morphol* 2004;**12**:160–5.
15 **Fischer G**, Odunsi K, Lele S, *et al.* Ovarian primary primitive neuroctodermal tumor (PNET) co-exisitng with endometrioid adenocarcinoma: a case report. *Int J Gynecol Pathol* (in press).
16 **Mager AM**, Grapin-Botton A, Ladjali K, *et al.* The avian fli gene is specifically expressed during embryogenesis in a subset of neutral crest cells giving rise to mesenchyme. *Int J Dev Biol* 1998;**42**:561–72.
17 **Sebenik M**, Ricci A, DiPasquale B, *et al.* Undifferentiated intimal sarcoma of large systemic blood vessels. Report of 14 cases with immunohistochemical profile and review of the literature. *Am J Surg Pathol* 2005;**29**:1184–93.
18 **Rossi S**, Orvieto E, furlanetto A, *et al.* Utility of IHC detection of FLI-1 expression in round cell and vascular neoplasm using a monoclonal antibody. *Mod Pathol* 2004;**17**:547–52.